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Full Length Article

Influence of Drought Stress on Growth, Ion Accumulation and Antioxidative Enzymes in Okra Genotypes

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ABSTRACT

The aim of this study was to investigate the effects of drought stress on plant growth, relative water content (RWC), ion concentration and activities of antioxidant enzymes, glutathione reductase (GR), and ascorbate peroxidase (APX) in 8 okra genotypes. Seeds were germinated in a mixture of peat: perlite of 2:1 ratio. After 16 days of sowing, seedlings were transferred to plastic pots containing mixture of peat: perlite of 2:1 ratio. Drought stress was achieved by decreasing the irrigation water gradually over 4 days (100%, 75%, 50% & 25%). There were considerable differences among the okra genotypes in their physiological responses to drought. Significant differences in response to drought were found to be closely related to differences in the potassium (K) and calcium (Ca) contents and the activities of antioxidant enzymes. Okra genotypes Okr-6, Okr-67, and Okr-105 showed higher antioxidant activity and higher K and Ca concentrations in the shoots and roots; therefore, were better able to perform better under drought stress. Whereas, genotypes Okr-47 and Okr-112 showed lower antioxidant activity and lower K and Ca concentrations and didn't perform well under drought. In conclusion, drought resistants genotypes exhibit a better protection mechanism against oxidative damage by maintaining a higher inherited and induced activity of antioxidant enzymes than the sensitive genotypes.

Key Words: *Abelmoschus esculentus*; Potassium; Calcium; Enzyme activity; Drought

INTRODUCTION

Drought stress is one of the most serious abiotic stresses that cause a reduction in plant growth, development, and yield in many parts of the world (Gong *et al*., 2005; Martinez *et al*., 2007; Kusvuran *et al*., 2011). However, plants have developed different morphological, physiological and biochemical mechanisms to withstand drought stress (Li *et al*., 2003; Farooq *et al*., 2009a & b). The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth. Understanding plant responses to drought is of great importance and also a fundamental part for making the crops stress tolerant (Jaleel *et al*., 2009).

Calcium (Ca) plays a vital role in many physiological processes such as membrane structure and stomatal function, cell division and cell wall synthesis, which influence growth and responses to environmental stresses. Moreover, it plays a significant role in osmoregulation. Under drought stress, potassium (K) increases the plant's drought resistance through its functions in stomatal regulation, osmoregulation, energy status, and charge balance. When water becomes limited, the plant generally experiences stomatal closure in an effect to prevent further water loss, limiting the carbon dioxide available for fixation by photosynthesis and reduces $NADP^+$ regeneration by the Calvin cycle (Kalefetoglu & Ekmekci, 2005). These converse conditions increase reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, singlet oxygen, and hydroxyl radicals (Turkan *et al.*, 2005; Yasar *et al*., 2008). These ROS attack lipids, proteins, and nucleic acids, causing lipid peroxidation (Kafkas *et al*., 2009), protein denaturing and DNA mutation. Plants possess several antioxidant enzyme systems that protect their cells from the negative effects of ROS. The role of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR)] as the components of the main tolerance mechanism developed in response to different stress conditions. Many reports suggest that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems and the relationship between enhanced or constitutive antioxidant enzyme activities and an increased resistance to drought stress (El-Tayeb, 2006; Liu *et al*., 2009; Basu *et al*., 2010).

Okra is grown around the world in various ecologies and it shows quite a genotypic variation. However, little is reported about the mechanism of drought resistance in okra. The present study was conducted to evaluate the mechanism of adaptation to drought stress. It was hypothesized that genotypes equipped with antioxidant defense are better able to withstand drought.

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MATERIAL AND METHODS

Eight okra genotypes viz. Okr-6, Okr-36, Okr-39, Okr-47, Okr-67, Okr-89, Okr-105 and Okr-112 were used in this study. Plants were grown in a growth chamber with day and night temperatures of approximately 26 and 19°C, respectively and relative humidity remained 60 to 70%. Seeds were germinated in a mixture of peat: perlite at a 2:1 ratio in the viol. After 16 days sowing, seedlings were transferred to plastic pots containing mixture of peat: perlite of 2:1 ratio.

In each pot, 2 plants were grown and 3 pots were included in each replicate. Following 1 week of growth in the pots, the seedlings were subjected to drought according to the procedure described by Kusvuran (2010). The applied amount of water in the study was calculated according to the ratio of "drained water/applied water". Under the control conditions, without stress, this ratio was approximately 30%. Drought stress was achieved by decreasing the irrigation water gradually over 4 days. The drought treatment started with pots that were 100% saturated and then the water deficit was performed at decrements of 25% (75%, 50% & 25%) of the control pots per day. At the end of the 4th day, the terminal water stress was started and irrigation was completely stopped (Kusvuran, 2010). The okra plants were subjected to drought stress for 10 days. The control plants were grown under non-stress conditions for the same period of time.

Responses of the okra genotypes to drought were evaluated using some plant growth and physiological parameters such as shoot fresh and dry weights, leaf number, leaf area, plant height and plant stem diameter (Kusvuran, 2010), relative water content (RWC) (Sanchez *et al*., 2004), shoot and root K and Ca contents (Dasgan & Koc, 2009) and GR and APX antioxidative enzyme activities (Cakmak & Marschner, 1992).

Determination of ion contents: At the end of the experiment, shoot and root were harvested and dried at 65ºC for 48 h and samples ashed at 550ºC and dissolved in 1% (v/v) HCl, analyzed for K and Ca by using an atomic absorption spectrometer (Varian Spectra AA 220 FS) (Dasgan & Koc, 2009).

Enzyme extraction and assay: Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80°C for further analyses. Enzymes were extracted from 0.5 g of leaf tissue using a mortar and pestle with 5 mL of extraction buffer containing 50 mM potassium phosphate buffer, pH 7.6, and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 × g for 15 min and the supernatant fraction was used to assay for the various enzymes. All of the steps in the preparation of the enzyme extracts were performed at 4°C.

APX activity was determined by measuring the consumption of ascorbate by following the absorbance at 290 nm. The amount of enzyme required to consume 1 µmole of ascorbate min^{-1} was defined as 1 unit of APX activity (Cakmak & Marschner, 1992).

GR activity was determined by measuring the enzyme-dependent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) by following the absorbance at 340 nm. The amount of enzyme that oxidized 1 µmole NADPH min^{-1} was defined as 1 unit of GR activity (Cakmak & Marschner, 1992).

The experiment was designed as a completely randomized plot with 3 replicates. Data were analyzed statistically, and the means of each treatment were analyzed by Duncan's multiple range tests using SAS software (Sas-Institute, 1985).

RESULTS

Drought stress decreased the plant growth parameter; shoot fresh weight (FW) and dry weight (DW), leaf number, leaf area, plant height and stem diameter in all of the okra genotypes. Shoot fresh and dry weights of the genotypes were decreased under drought stress (Table I). The genotypes responses were significantly different. Some okra genotypes protected their biomass growth, while others were more affected. Okr-6 was the least affected, losing 38% in FW and 22% in DW. However, genotype Okr-89 was the most affected, losing 71% in FW and 81% in DW under drought stress. The other genotypes showed different rates of weight loss, ranging between that of these 2 genotypes (Table I). Okr-36 and Okr-105 showed some protection of their fresh and dry weights; however, Okr-47 did not.

Plant height decreased under drought stress. Genotypes Okr-89 and Okr-105 were the least affected, with decreases of 10% and 7%, respectively. However, genotypes Okr-47 and Okr-112 were the most affected, with decreases of 25% and 23%, respectively (Table I).

Plant stem diameters also decreased under drought stress. Genotypes Okr-6 and Okr-105 showed the least reduction in plant height, with decreases of 27% and 23%, respectively. However, genotypes Okr-67 and Okr-112 showed the most reduction in plant height, with decreases of 38% and 41%, respectively (Table I).

Drought stress reduced the leaf area and leaf number in all of the okra genotypes studied (Table II). The leaf number reduction under drought stress was different among the okra genotypes. The least reductions were in genotypes Okr-6 and Okr-105, with decreases of 14% and 10%, respectively. However, genotypes Okr-39, Okr-47, and Okr-89 showed important leaf reductions, with decreases of 28%, 32%, and 26%, respectively. Plant leaf area under drought stress was also significantly affected. The least reductions were in genotypes Okr-6 and Okr-105, with a decrease of 35% in both genotypes. However, genotypes Okr-47 and Okr-112 showed serious reductions in leaf area, with decreases of 82% and 76%, respectively.

The leaf RWC was decreased under drought stress. Among the genotypes, Okr-67, Okr-89, and Okr-105 protected their RWC, while genotypes Okr-47 and Okr-112

were decreased by 22% and 19%, respectively compared with the control plants (Table II).

In the control plants without stress, K concentrations were significantly higher in the shoots and roots than in the drought stressed plants; however, under drought, the K contents in all of the genotypes were higher in the shoots than in the roots. Genotypes Okr-6, Okr-67 and Okr-105 protected their K concentrations and decreased only 20%, 19% and 31%, respectively (Table III).

The drought application caused a significant reduction in Ca concentrations in the shoots and roots of the okra genotypes. The reduction percentage was between 25% and 53% in the shoots and between 6% and 27% in the roots. Ca contents in the shoots and roots of Okr-6 and Okr-105 were greater than those of the other genotypes under stress conditions (Table III). A significant decrease in Ca

Table I: The effects of drought stress on fresh and dry shoot weights, plant height and plant stem diameter in the okra genotypes

	Fresh Weight (g/plant)		Dry Weight (g/plant)		Plant Height (cm/plant)		Stem diameter (mm/plant)	
No	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Okr-6	10.66 b	6.63 c	1.01 de	0.79 ef	17.69 b-d	15.18 ef	3.65 cd	2.66 g
Okr-36	10.69 b	6.06 cd	1.50 c	0.73 ef	18.63 bc	15.31 ef	4.12 bc	3.06 e-g
Okr-39	15.40 a	5.37 cd	1.94 b	0.65 fg	23.19 a	18.00 bc	4.87 a	3.21 d-f
Okr-47	14.60 a	4.88 cd	2.21 ab	0.57 fg	15.33 ef	11.55 g	4.06 bc	2.75 fg
Okr-67	15.30 a	5.42 cd	2.11 ab	0.64 fg	19.25 b	15.93 de	5.16 a	3.18 d-f
Okr-89	13.75 a	3.93 d	1.88 a	0.35 g	18.38 bc	16.62 c-e	3.93 bc	2.59 g
Okr-105	12.98 ab	6.00 cd	1.34 cd	0.82 ef	17.06 c-e	15.93 de	4.31 b	3.30 de
Okr-112	14.04 a	5.50 cd	2.33 a	0.67 e-g	17.65 b-d	13.37 fg	4.90 a	2.96 e-g
LSD(0.05)	2.56		0.35		2.03		0.51	

Table II: The effects of drought stress on the leaf number, leaf area, and RWC in the okra genotypes

	Leaf Number (number/plant)		Leaf Area (cm^2/plant)		RWC (%)	
No	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Okr-6	5.00 b-e	4.28 e-h	203.77 d	132.97 ef	89.61 ab	76.14 d
Okr-36	5.62 a-c	4.75 c-f	295.53 bc	100.46 f-h	92.08 ab	80.41 cd
Okr-39	5.88 ab	4.25 e-h	304.36 b	98.32 f-h	92.13 ab	77.19 d
Okr-47	5.89 ab	4.12 e-h	363.87 a	65.19 h	89.21 ab	69.79 e
Okr-67	4.62 d-g	3.75 gh	309.26 b	102.49 fg	88.93 ab	78.52 d
Okr-89	4.75 c-f	3.50 h	266.97 c	92.85 gh	91.37 ab	86.57 bc
Okr-105	6.12 a	5.50 a-d	217.69 d	141.95 e	91.33 ab	80.44 cd
Okr-112	5.43 a-d	4.00 f-h	309.90 b	79.31 gh	93.11 a	75.37 d
LSD(0.05)	0.88		35.90		6.25	

Table III: The effects of drought stress on K and Ca concentrations in the shoots and roots of the okra genotypes

	Plant K (%)		Root K (%)		Plant Ca (%)		Root Ca (%)	
No	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Okr-6	3.15 c	2.52 d	2.25 de	1.96 e	3.49 a	2.44 d-f	2.36 ab	2.26 a-c
Okr-36	3.30 c	2.15 d	2.77 bc	2.16 de	3.39 ab	2.16 fg	2.10 b-e	1.76 ef
Okr-39	3.53 bc	2.06 d	2.93 ab	2.53 b-d	2.91 b-d	2.14 fg	2.14 a-e	1.71 f
Okr-47	4.26 a	1.99 d	2.85 ab	1.85 e	3.53 a	1.60 h	2.52 a	1.81 d-f
Okr-67	4.08 ab	3.32 c	2.99 ab	2.65 b-d	3.28 ab	2.26 e-g	2.28 a-c	1.91 c-f
Okr-89	3.72 a-c	2.20 d	3.32 a	2.33 c-e	3.14 a-c	1.81 gh	2.17 a-d	1.69 f
Okr-105	3.29 c	2.26 d	2.82 b	2.23 de	3.66 a	2.72 c-e	2.11 b-e	1.98 b-f
Okr-112	4.13 a	1.94 d	2.77 bc	2.24 de	2.91 b-d	2.03 f-h	1.86 d-f	1.78 ef
LSD(0.05)	0.59		0.48		0.54		0.38	

Table IV: The effects of drought stress on GR and APX enzyme activities of the okra genotypes

	GR Activity (µmol/min/mg FW)			APX Activity (µmol/min/mg FW)		
No	**Control**	**Drought**	**Difference (%)**	**Control**	**Drought**	**Difference (%)**
Okr-6	5.72 h	13.40 bc	136.01	2.66 gh	10.84 bc	307.52
Okr-36	6.10 h	13.40 b-d	119.67	3.81 g	12.43 a	226.25
Okr-39	6.68 gh	14.29 b	113.92	2.88 gh	11.95 ab	314.93
Okr-47	8.49 fg	11.14 de	31.21	2.74 gh	6.67 f	143.43
Okr-67	6.49 gh	14.42 b	122.19	2.43 h	10.27 cd	322.63
Okr-89	5.63 h	11.45 c-e	103.55	2.33 h	8.99 de	285.84
Okr-105	7.06 gh	19.29 a	173.23	2.95 gh	13.27 a	349.83
Okr-112	8.31 fg	10.41 ef	25.27	3.04 gh	8.61 e	183.22
LSD(0.05)	2.16			1.37		

*Mean values indicated by the same letter(s) in same column are not significantly different ($P < 0.05$)

concentrations was observed in Okr-47 and Okr-112 (Table III).

There were striking differences in antioxidant enzyme activities among the genotypes under drought stress. GR activity increased under drought stress in all of the genotypes when compared to the controls (Table IV). The rate of increase was higher in Okr-6 and Okr-105, with 136% and 173%, respectively (Table IV). The lowest rates of increase were found in Okr-47 and Okr-112, with 31% and 41%, respectively (Table IV).

APX activity in the okra genotypes increased under drought stress (Table IV). Among the genotypes, Okr-67 and Okr-105 showed the highest increases, with 322% and 349%, respectively (Table IV). However, in genotypes Okr-47 and Okr-112, the APX activity increased only 136% and 196%, respectively in comparison to the controls (Table IV).

DISCUSSION

Drought stress is the most important problem in agriculture. Plants adapt to drought through various physiological and biochemical strategies. In this study, a substantial drought-induced alteration in plant growth of okra genotypes was observed. This differential growth of okra genotypes may have been due to differential regulation of physiological and biochemical properties involved under drought stress.

Growth inhibition (shoot fresh & dry weight, plant height, leaf area & leaf number) was the first major symptomatic effect under drought stress. Hessini *et al*. (2009) indicated that, the decrease in total biomass production was mainly associated to a reduction of the leaf biomass produced by a reduction of leaf area, transpiration and photosynthesis. Jaleel *et al.* (2009) reported to development of optimal leaf area is important to photosynthesis and dry matter yield. Moreover, leaf number and leaf area reduced by water deficit stress. In the present study, resistant genotypes (Okr-6, Okr-67 & Okr-105) protected their fresh and dry weights compared to sensitive ones (Okr-47 & Okr-112). This strongly suggests that drought stress reduced leaf growth and leaf area in sensitive genotypes. Consequently, those plants with a lower transpiration could have a lower biomass accumulation. Similar results were obtained by Yong *et al*. (2006), Sankar *et al*. (2008), Kusvuran *et al*. (2008); Kusvuran (2010) and Sanchez-Rodriguez *et al*. (2010).

Decrease in plant height by drought may be due to a decrease in cell elongation and differentiation. There are some reports that plant height and diameter were significantly affected under water deficiency in okra (Sankar *et al*., 2007; Kusvuran *et al*., 2008); wheat (Abdalla & El-Khoshiban, 2007) and melon (Kusvuran, 2010).

RWC indicates the water status of a plant and is amongst the important indicators of water stress in leaves (Dhanda & Sethi, 2002; Hessini *et al.*, 2009) In this study, among the genotypes, Okr-6, Okr-36 and Okr-105 maintained RWC; however, sensitive genotypes, Okr-47 and Okr-112, showed the lowest RWC. Moussa and Abdel-Azizi (2008) reported that the high RWC may be helpful to continue physio-biochemical processes efficiently under water stress conditions. However, genotype of wheat Khanna-Chopra and Selote (2007) and in tomato Sanchez-Rodriguez *et al*. (2010) strongly differs in this regard.

Under drought, the K contents in leaf and root were decreased in all genotypes; however extent of decrease was less in resistant genotypes than sensitive genotypes. Higher uptake and accumulation of K under drought is regarded as a better strategy to cope with drought. Potassium plays an important role in regulating the stomatal oscillations and osmoregulation under drought in particular (Nasri *et al*., 2008; Dasgan & Koc, 2009; Kusvuran, 2010). According to Cakmak (2005), increases in severity of drought stress result in corresponding increase in K demand to maintain photosynthesis and protect chloroplasts from oxidative damage. In this study, drought resistance genotypes may be accumulated more K in their tissue than sensitive genotypes and protected oxidative damage.

Likewise, drought decreased the Ca concentrations in the shoots and roots. Ca uptake and rates differed among the genotypes under drought stress. The most significant decreases in Ca concentrations were observed in the drought-sensitive genotypes Okr-47 and Okr-112. However, the drought-resistant okra genotypes retained the highest Ca content when compared to the drought-sensitive genotypes. Water deficiency causes a decrease in the flow of nutrients to stem cells from other tissues and organs, and such a decrease leads to the occurrence of nutrient deficiencies in different tissues. Water scarcity leads to a decrease in the concentration of Ca in tissues. Ultimately, Ca ion transport mobility is restricted in the xylem and phloem (Kiegle *et al*., 2000). This decreases certain metabolic processes such as photosynthesis, respiration and enzyme activities that occur outside of the disruptions due to a decrease in membrane permeability and osmotic balance and plant growth is blocked (Dasgan & Koc, 2009). Therefore, the maintenance of Ca acquisition and transport under water stress is an important determinant of drought resistance (Saxena & Nautiyal, 2001).

The generation of ROS is a common response to environmental stress conditions (Moussa & Abdel-Azizi, 2008; Farooq *et al.*, 2009a, b; Kusvuran, 2010). One consequence of drought stress in plants is excessive generation of ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals (Foyer *et al.*, 1994; Moussa & Abdel-Azizi, 2008; Kusvuran, 2010). Under normal growth conditions, the production of ROS in the cell is generally at low levels. However, under conditions of abiotic stress such as severe drought, cellular homeostasis is disrupted and leads to the production of relatively high levels of ROS (Yasar *et al*., 2006). These radicals can damage vital cellular macromolecules (e.g., via denaturation of proteins, mutation of DNA and/or peroxidation of lipids). Plants have evolved

both enzymatic and non-enzymatic mechanisms to scavenge ROS (Asada, 1999).

GR plays a key role in the response to oxidative stress, by maintaining the intracellular glutathione pool mainly in the reduced state an especially functions as an antioxidant that scavenges ROS such as hydrogen peroxide and superoxide (Yousuf *et al*., 2012). Turkan *et al*. (2005) also found that GR activity could be important in protection against oxidative stress. In this study, the okra genotypes responded to drought stress by increased GR activity. On the other hand, in the drought- resistance genotypes, GR activity more increased significantly in the drought conditions than sensitive genotypes. This indicates that drought resistance genotypes induced capability of plant protection against oxidative damage caused by drought stress. Increased in GR activity have been reported to play a role in resistance to drought stress in mulberry (Reddy *et al*., 2004), maize (Wang *et al*., 2008); melon (Kusvuran, 2010).

Increases in APX activity have been reported to play a role in resistance to *Radix Astragali* (Yong *et al*., 2006), tomato (Sanchez-Rodriguez *et al*., 2010) and almod (Sorkheh *et al*., 2010). Turkan *et al*. (2005) also observed that drought increased APX activity in drought-tolerant plants. Similarly, in this study, APX activity in drought-tolerant Okr-105 and Okr-67 were increased compared with the other genotypes. These results suggest that APX activity may play a significant role in the destruction of ROS, such as H_2O_2, in drought resistance genotypes.

In conclusion, there were considerable differences among the okra genotypes in their physiological responses to drought stress. Significant differences in response to drought were found to be closely related to differences in K and Ca contents and the activities of antioxidant enzymes. Overall findings suggest that okra genotypes Okr-6, Okr-67 and Okr-105 showed higher antioxidant activity and higher K and Ca concentrations in the shoots and roots. Increase in resistance to drought stress is associated with antioxidative enzyme activities, and that antioxidative defense mechanisms were effective in providing resistance to drought stress in okra genotypes.

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